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(54) **Pharmaceutical preparations containing tumor cytotoxic factor**

Einen für Tumore cytotoxischen Faktor enthaltende pharmazeutische Zusammensetzung

Préparations pharmaceutiques contenant le facteur cytotoxique tumoral

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- **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol.88, 1991, WASHINGTON US pages 7001 - 7005 WEIDNER K.M. ET AL 'Evidence for the identity of human scatter factor and human hepatocyte growth factor'**

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Description

Field of the Invention

[0001] This invention relates to pharmaceutical preparations having tumor cytotoxic factor activity.

Background of the Invention

[0002] Tumor cytotoxic factor, hereinafter abbreviated as TCF, is an another name of TCF-II found in a cultured supernatant of human fibroblast cells and disclosed in WO 90/10651. TCF is a glycoprotein consisting of heterodimer having molecular weight of about 76-80 kDa in unreduced state, and α subunit having molecular weight of about 52-56 kDa and β or β' subunit having molecular weight of about 30-36 kDa in reduced state.

[0003] TCF exhibits various biological activities such as the activities of hepatocyte growth factor; HGF, scatter factor; SF, proliferation factor of renal tubular epithelial cells, repair factor for damaged tissues and proliferation factor of vascular endothelial cells, in addition to TCF activity. TCF is a cytokine belonging to a member of HGF family. TCF is expected to be developed as pharmaceuticals for the treatment of diseases of liver and kidneys, wounds and tumors due to its various kinds of physiological activity.

[0004] However, the solubility in water of TCF is very low. Thus aqueous preparations such as injections with high concentrations satisfying medical use are hardly obtained. Therefore, one of the most serious subject to be solved for its application to the clinical use is preparing such a concentrated solution of TCF. TCF has rapid metabolic turnover in vivo and high dosage is expected on the clinical use. The clinical dosage of TCF is expected to be 1-10 mg/day for adult patients. To make sure of the quality of final products, such as injections, the production processes require to dissolve TCF at high concentrations and to mix it with additives such as stabilizers under low temperatures. Furthermore, a highly concentrated TCF solution is demanded for medical treatment, that is the solution of neutral pH and has isotonicity for injections. No such method to prepare highly concentrated TCF solution has been developed yet. For example, an isotonic saline solution, containing 0.15 M sodium chloride usually used for injectins, dissolves TCF less than 5 mg/ml and the TCF solution is unstable. The solubility of TCF decreases and TCF becomes insoluble with the progress of time at room temperature. Furthermore, solubility of TCF in saline markedly decreases to about 1 mg/ml at 5 °C or lower.

[0005] Therefore, it is an important subject to establish a method for preparing a neutral, isotonic and highly concentrated TCF solution at low temperatures. Above mentioned WO 90/10651 discloses preparations containing a protein, a sugar, an amino acid and so forth as an adsorption preventive agent or a stabilizer. However, neither highly concentrated TCF solution of the present invention is disclosed nor suggested.

[0006] The present invention aims to provide a highly concentrated and isotonic TCF injection solutions for medical treatment.

Brief Description of the Drawings

[0007]

Fig. 1 shows a stability on storage of the TCF solution prepared by adding sodium chloride as an dissolution adjubant.

Fig. 2 shows a stability on storage of the TCF solution prepared by adding L-arginine hydrochloride and sodium chloride as dissolution adjuvants and D-mannitol as a stabilizer.

Fig. 3 shows a stability on storage of the TCF solution prepared by adding sodium chloride as an dissolution adjuvant, and human serum albumin (HSA) and D-mannitol as stabilizers.

Summary of the Invention

[0008] The inventors have been investigating to overcome the difficulty in dissolving TCF at high concentrations and found the following characteristic feature of solubility of TCF and accomplished the present invention.

(1) TCF shows temperature dependent solubility in water.
(2) TCF exhibits higher solubility at lower pH regions under a pH range of 5-8.
(3) Addition of a salt such as sodium chloride, preferably at concentrations of 0.3 M or over, provides markedly increased solubility of TCF.
(4) Addition of a basic amino acid, preferably 1.0-4.0 % of arginine or lysine, markedly elevates the solubility under neutral pH and isotonicity of about 300 mOsm.

[0009] The present invention provides a TCF preparation comprising at least 5 mg/ml TCF and a basic amino acid or a salt of a basic amino acid.

[0010] The present invention further provides an isotonic injectable TCF preparation having an approximately neutral pH comprising at least 5 mg/ml TCF and a basic amino acid or salt of a basic amino acid.

[0011] The present invention can provide preparations of TCF solution with improved solubility to satisfy the use for medical treatments.

[0012] The present invention can provide highly concentrated TCF solution containing one or more solubilizing agents selected from the group consisting of basic amino acids or their salts, and these amino acids together with pharmacologically acceptable organic or inorganic salts.

[0013] The present invention can provide highly concentrated TCF injections with neutral pH and isotonicity containing one or more solubilizing agents selected from the group consisting of basic amino acids or their salts, and these amino acids together with pharmacologically acceptable organic or inorganic salts.

Detailed Description of the Invention

[0014] The TCF pharmaceuticals of the present invention contain 5 mg/ml or over TCF together with a basic amino acid and/or inorganic or organic salt as solubilizing agent(s) at concentrations to give isotonic solution. The pharmaceuticals of the present invention must be homogenous mixtures of TCF and the solubilizing agent(s) in the case of dissolving them before use. To prepare these pharmaceuticals, the TCF solution at the desired concentration must be prepared and divided in vials or ampoules, occasionally lyophilyzed, and sealed. Highly concentrated TCF solutions are essential to prepare these pharmaceuticals. However, the solubility of TCF in water is very low. Acidic condition of pH 6 or less, or ionic strength of 0.3 M or over of sodium chloride is required to increase the solubility of TCF. But acidic injections cause patients pain on injection and are unpreferable. Also, a higher concentration of sodium chloride is unpreferable because of raising osmotic pressure of the injections.

[0015] To maintain isotonicity, one may prepare 0.15 M sodium chloride solution having osmotic pressure of about 300 mOsm. However, this solution dissolves only about 1 mg/ml of TCF at low temperature. To dissolve TCF up to about 10 mg/ml, 0.3 M or over sodium chloride solution is required, but its osmotic pressure becomes 600 mOsm or over. Therefore, multiple solubilizing agents are required to obtain the solution dissolving TCF at 10 mg/ml or over on the isotonic condition.

[0016] As solubilizing agents, basic amino acids and organic or inorganic salts and both of them can be used. Arginine or lysine is preferable as a basic amino acid and their salts also be used. Solutions containing these amino acids or their salts at concentrations of 3-4 % of as free amino acid are almost isotonic. These solutions can dissolve TCF at concentrations of 10-20 mg/ml. Furthermore, these basic amino acids or their salts may be combined with one or more organic and inorganic salts. For examples, 1.5-1.75 % of the amino acid solution with pharmacologically acceptable organic and inorganic salts can be isotonic. Sodium citrate or sodium lactate may be exemplified as organic salts. Sodium chloride, disodium hydrogenphosphate or sodium hydrogencarbonate may be exemplified as inorganic salts, and sodium chloride is preferable. TCF dissolves at concentration of 5-10 mg/ml by using the basic amino acid with the salt. This TCF solution can be used as injections after being sterilized, divided in vials or ampoules and sealed. Also the solution may be freeze-dried to give lyophilized pharmaceuticals. Lyophilized pharmaceuticals may be preparaed by dissolving TCF at twofold concentration into a solution containing a basic amino acid and a salt at two times concentration (e.g. 7 % or higher basic amino acid or a combination of 4 % of basic amino acid and 0.15 M sodium chloride) and lyophilizing, as TCF easily dissolves at a high concentration in a solution of a basic amino acid and a salt. This procedure may reduce time and energy required for lyophilization. The lyophilized preparations may be re-dissoleved in twofold distilled water for injection to give isotonic intramuscular or intravenous injections. The TCF pharmaceuticals or injections of the present invention include these lyophilized pharmaceuticals.

[0017] As TCF is easily adsorbed to glass or synthetic resins, addition of a surfactant, an adsorption preventive agent or a stabilizer may be helpful to prevent TCF from adsorping. Tween 20, Tween 80 and Tween 100 can be exemplified as surfactat. Human serum albumin, gelatin, sorbitol, mannitol and xylitol disclosed in WO 90/10651 may be exemplified as adsorption preventive agents and stabilizers.

[0018] The TCF pharmaceutical preparations of the present invention can be stored for a long period of time maintaining sufficient amount of TCF for the treatment of diseases which require higly concencentrated solution of TCF.

[0019] The present invention will be explained by the following examples and reference examples. The present invention, however, is not restricted by these examples.

[Examples]

[0020] The present examples show the preparation of highly concentrated pharmaceuticals of TCF.

[0021] TCF used for examples, reference examples and experiments is recombinant TCF (r-TCF) produced by

genetically engineered Namalwa cells by application of recombinant DNA technique according to the method disclosed in WO 92/1053. Solutions containing TCF were prepared with 10 mM phosphate buffer containing 0.01 % Tween 80 as an adsorption preventive agent.

(1) A preparation of injections containing TCF at high concentration

[0022]

① TCF solution was prepared by dissolving TCF to 20 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 0.01 % of Tween 80 and 0.3 M of sodium chloride.
② An aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 2.33 % L-arginine hydrochloride and 0.01 % Tween 80 was prepared. This solution and the TCF soliution prepared in ① were mixed at a ratio of 3:1. After the solutions were mixed well, the mixed solution was sterilized with a filter having 0.22 µm pores and divided 1 ml each in ampoules and sealed. The prepared solution showed neutral pH and isotonicity and contained 5 mg/ml of TCF, 0.075 M of sodium chloride and 1.75 % L-arginine hydrochloride. Therefore this solution is most preferable for injections as pharmaceuticals. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

(2) A preparation of injections containing TCF at high concentration

[0023] TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 3.5 % of DL-arginine hydrochloride and 0.01 % of Tween 80. This TCF solution was sterilized with the filter and divided 1 ml each to vials and sealed. The solution, showed neutral pH and isotonicity and contained 10 mg/ml of TCF, is most preferable for injections. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

(3) A preparation of injections containing TCF at high concentration

[0024] TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 3.0 % of L-lysine hydrochloride and 0.01 % of Tween 80. This TCF solution was sterilized with the filter and divided 1 ml each to vials and sealed. The solution, shows neutral pH and isotonicity and contains 10 mg/ml of TCF, is most preferable for injections. Furthermore, the solution is stable without becoming turbid and maintains the initial concentration of TCF at room temperature or lower.

(4) A preparation of lyophilized injections containing TCF at high concentration

[0025] TCF was dissolved to 10 mg/ml in an aseptic pyrogen-free 10 mM phosphate buffer (pH 7) containing 7.0 % of DL-arginine hydrochloride and 0.02 % of Tween 80. This TCF solution was sterilized with the filter, divided 1 ml each to vials, lyophilized and sealed. The lyophilized preparation was re-dissolved in 2 ml of distilled water for injection before use to give 5 mg/ml solution of TCF which showed neutral pH and isotonicity.

[Experiment 1]

[0026] The solubility test of TCF is explained by the following test experiments. The present test gave findings concerning the profile of TCF solubility required for preparaing pharmaceuticals of TCF.

(1) Evaluation of solubility of TCF

[0027] TCF was weighed in polypropylene tubes, and solutions of various pHs containing various concentrations of sodium chloride and/or an amino acid were added into the tubes. To dissolve TCF, the tubes were immediately placed at a constant temperature and stirred for 30 min. according to the method described in the XIIth Pharmacopoeia of Japan (JP XII), General notices 24. The tubes were ultracentrifuged at 30,000 x g for 30 min. at a constant temperature to separate unsolved TCF. The concentration of TCF in the obtained saturated TCF solutions was measured by Lowry-Folin's method to determine solubility of TCF.

(2) Effect of pH on solubility of TCF

[0028] Solutions of various pHs containing 0. 15 M sodium chloride or not were prepared. Solubilities of TCF in

these solutions were determined at 5°C and 20°C according to the method shown in experiment (1) and the results are shown in Table 1. The results showed that the solubilitiies of TCF were increased depending on the decline of pH at pH 7 or lower.

[Table 1]

pH	0 M NaCl	0. 15 M NaCl	
	20 °C	5 °C	20 °C
5.5	1.9	5.6	15.0
6.0	1.0	2.8	12.4
6.5	0.6	1.7	6.5
7.0	0.4	1.2	4.9
7.5	-	-	4.6
8.0	-	-	4.8

* Solubility is expressed as mg/ml.

(3) Effect of concentration of sodium chloride on solubility of TCF

[0029] Solutions of various concentration of sodium chloride at pH 6, pH 6.5 and pH 7 were prepared. Solubilities of TCF in these solutions were determined at 20 °C according to the method shown in experiment (1) and the results are shown in Table 2. The results showed the remarkable increse of solubility of TCF when the concentration of sodium chloride was raised from 0.15 M to 0.3 M. However, the increase of concentration of sodium chloride from 0.3 M to 1.2 M made a slight raise of the solubility of TCF.

[Table 2]

Concentration of sodium chloride (M)	pH		
	6.0	6.5	7.0
0	1.0	0.6	0.4
0.15	12.4	6.5	4.9
0.3	53.7	51.4	49.4
1.2	62.1	57.5	53.9

* Solubility is expressed as mg/ml.

(4) Effect of temperature on solubility of TCF

[0030] Solutions containing 0.15 M or 0.3 M sodium chloride at pH 6, pH 6.5 and pH 7 were prepared. Solubilities of TCF in these solitions were determined at different temperatures according to the method shown in experiment (1) and the results are shown in Table 3. The results showed the remarkable increase of solubility of TCF in temperature dependent manner.

[Table 3]

Temperature (°C)	0.15 M NaCl			0.3 M NaCl		
	pH 6.0	pH 6.5	pH 7.0	pH 6.0	pH 6.5	pH 7.0
5	2.8	1.7	1.2	50.6	39.2	37.0
20	12.4	6.5	4.9	53.7	51.4	49.4
40	32.9	31.1	30.0	60.7	59.7	58.7

* Solubility is expressed as mg/ml.

(5) Effect of solubilizer on solubilitiy of TCF

[0031] In consideration of physiological conditions to use TCF for pharmaceuticals, solutions of neutral pH of 6.8-7.2 for dissolving TCF were prepared using various amino acids as solubizers and sodium chloride to adjust osmotic pressure to about 300 mOsm. The solubility of TCF was determined at 5 °C according to the method shown in experiment (1) and the results are shown in Table 4.

[Table 4]

Amino acid	Concentration	0 M NaCl	0.075 M NaCl	0.15M NaCl
-	-	-	-	1.2
Gly	2 %	1.0	-	-
	1 %	-	3.0	-
L-Ala	2.5 %	1.5	-	-
	1.25 %	-	3.2	-
L-Ser	3 %	1.0	-	-
	1.5 %	-	1.9	-
L-Met	4 %	1.2	-	-
	2 %	-	2.0	-
L-Pro	3 %	1.4	-	-
	1.5 %	-	3.0	-
L-Asp • Na • H_2O	3 %	5.3	-	-
	1.5 %	-	3.4	-
L-Glu • Na • H_2O	3 %	4.7	-	-
	1.5 %	-	3.3	-
L-Arg • HCl	3.5 %	18.4	-	-
	1.75 %	-	7.6	-
D-Arg • HCl	3.5 %	21.7	-	-
	1.75 %	-	10.3	-
DL-Arg • HCl	3.5 %	21.7	-	-
	1.75 %	-	8.5	-
L-Lys • HCl	3 %	10.4	-	-
	1.5 %	-	6.9	-
L-His	4 %	3.5	-	-
	2 %	-	3.2	-

* Solubility is expressed as mg/ml.

[0032] Neutral amino acids such as glycine at concentration of 1-4 % gave slight increase of solubility and acidic amino acids such as sodium L-aspartate monohydrate at concentration of 1-5 % incresed the solubility about 3-4 times than that in the solution with no amino acid. But significant solubilizing effect of these amino acids were not found.

[0033] On the contrary, basic ammino acids such as L-arginine at concentration of 1.75-3.5 % significantly increased solubility of TCF.

[0034] The solubilities were 5-15 times higher than that of no addition of amino acid. Furthermore, L-, D- and DL-forms of arginine gave same results. L-lysine at concentration of 1.5-3 % also gave increase of solubility. However, L-histidine at concentration of 2-4 % showed only about 3 times higher solubilities than that of no addition of amino acid.

[0035] The present invention can provide TCF pharmaceuticals of 10 mg/ml or more concentrations using basic amino acids, sodium chloride and so forth as a solubilizing agent. On the contrary, TCF dissolves at a concentration of about 1 mg/ml in neutral and isotonic solution without the solubilizing agent.

[Experiment 2]

[0036] Basic amino acids used in the present invention are effective to improve the stability of TCF during storage.

The effect is explained by the following test experiments which show the effects of various additives on the stability of TCF during storage.

[0037] Human serum albumin (HSA) and D-mannitol were added to the solutions containing sodium chloride and/or L-arginine hydrochloride. TCF was dissolved at concentrations of 1 mg/ml in these solutions at room temperature. The prepared TCF solutions were sterilized with a filter having pore size of 0.22 μm and divided in polypropylene tubes. The tubes were kept at 5 °C or 20°C for 1, 4 and 7 days. The tubes were ultracentrifuged by the method described in Experiment (1). The concentration of TCF in the supernatant was measured by the method of Lowry-Folin and enzyme-linked immunosorbent assay (ELISA) disclosed in Japanese Un-examined Patent Publication No. 97 (1993), and residual TCF was caluculated. The effects of the additives on the solubility of TCF were evaluated.

[0038] The results are shown in Fig. 1, 2 and 3. The values are expressed as percentage to the initial amount of TCF. As shown in the figures, L-arginine hydrochloride increased the stability of the pharmaceuticals of the present invention.

Claims

1. A tumour cytotoxic factor (TCF) preparation comprising:

   at least 5 mg/ml TCF;
   a basic amino acid or a salt of a basic amino acid.

2. An isotonic injectable TCF preparation having an approximately neutral pH comprising:

   at least 5 mg/ml TCF;
   a basic amino acid or salt of a basic amino acid.

3. The preparation of claim 1 or 2 further comprising a pharmacologically acceptable organic or inorganic salt.

4. The preparation of claim 1, 2 or 3 wherein the concentration of TCF is at least 10 mg/ml.

5. The preparation of any preceding claim wherein the basic amino acid or salt is lysine or arginine, or a salt of lysine or arginine.

6. The preparation of any preceding claim wherein the basic amino acid or salt thereof is present in an amount sufficient to enhance solubility of TCF at approximately neutral pH.

7. The preparation of any preceding claim wherein the pharmacologically acceptable salt, if present, is sodium citrate, sodium lactate, disodium hydrogen phosphate or sodium hydrogen carbonate.

8. The preparation of any preceding claim wherein the pharmacologically acceptable salt, if present, is sodium chloride.

9. The preparation of any preceding claim which also contains a surfactant, an adsorption preventing agent or a stabilizer or a combination of any two or more thereof.

10. A method for producing the preparations of claims 1-9, the method comprising combining TCF with a basic amino acid or salt thereof.

11. The method of claim 10 in which the basic amino acid or salt is lysine or arginine, or a salt of lysine or arginine.

12. The method of claim 11 in which the pharmacologically acceptable salt, if present, is sodium citrate, sodium lactate, disodium hydrogenphosphate, sodium hydrogencarbonate or sodium chloride.

Patentansprüche

1. TCF- (tumorzytotoxischer Faktor) -Präparat, umfassend:

   wenigstens 5 mg/ml TCF;
   eine basische Aminosäure oder ein Salz einer basischen Aminosäure.

2. Isotonisches injizierbares TCF-Präparat mit einem in etwa neutralen pH-Wert, umfassend:

   wenigstens 5 mg/ml TCF;
   eine basische Aminosäure oder ein Salz einer basischen Aminosäure.

3. Präparat nach Anspruch 1 oder 2, ferner umfassend ein pharmakologisch akzeptables organisches oder anorganisches Salz.

4. Präparat nach Anspruch 1, 2 oder 3, bei dem die Konzentration von TCF wenigstens 10 mg/ml beträgt.

5. Präparat nach einem der vorherigen Ansprüche, bei dem die basische Aminosäure oder das Salz Lysin oder Arginin oder ein Salz von Lysin oder Arginin ist.

6. Präparat nach einem der vorherigen Ansprüche, bei dem die basische Aminosäure oder ein Salz davon in einer Menge anwesend ist, die ausreicht, um die Löslichkeit von TCF bei etwa neutralem pH-Wert zu verbessern.

7. Präparat nach einem der vorherigen Ansprüche, bei dem das pharmakologisch akzeptable Salz, sofern vorhanden, Natriumcitrat, Natriumlactat, Dinatriumhydrogenphosphat oder Natiumhydrogencarbonat ist.

8. Präparat nach einem der vorherigen Ansprüche, bei dem das pharmakologisch akzeptable Salz, sofern vorhanden, Natriumchlorid ist.

9. Präparat nach einem der vorherigen Ansprüche, das außerdem ein Tensid, ein Adsorptionspräventivmittel oder einen Stabilisator oder eine Kombination von zweien oder mehreren davon enthält.

10. Verfahren zur Herstellung der Präparate nach den Ansprüchen 1-9, wobei das Verfahren das Kombinieren von TCF mit einer basischen Aminosäure oder einem Salz davon umfasst.

11. Verfahren nach Anspruch 10, bei dem die basische Aminosäure oder das Salz Lysin oder Arginin oder ein Salz von Lysin oder Arginin ist.

12. Verfahren nach Anspruch 11, bei dem das pharmakologisch akzeptable Salz, sofern vorhanden, Natriumcitrat, Natriumlactat, Dinatriumhydrogenphosphat, Natriumhydrogencarbonat oder Natriumchlorid ist.

Revendications

1. Préparation de facteur cytotoxique tumoral (TCF) comprenant :

   au moins 5 mg/ml de TCF;
   un acide aminé basique ou un sel d'un acide aminé basique.

2. Préparation isotonique injectable de TCF ayant un pH approximativement neutre comprenant :

   au moins 5 mg/ml de TCF;
   un acide aminé basique ou sel d'un acide aminé basique.

3. Préparation de la revendication 1 ou 2 comprenant en outre un sel organique ou inorganique pharmacologiquement acceptable.

4. Préparation de la revendication 1, 2 ou 3 dans laquelle la concentration de TCF est au moins de 10 mg/ml.

5. Préparation de l'une quelconque des revendications précédentes dans laquelle l'acide aminé basique ou sel est de la lysine ou de l'arginine, ou un sel de lysine ou d'arginine.

6. Préparation de l'une quelconque des revendications précédentes dans laquelle l'acide aminé basique ou sel de celui-ci est présent en une quantité suffisante pour mettre en valeur la solubilité du TCF à un pH approximativement neutre.

7. Préparation de l'une quelconque des revendications précédentes dans laquelle le sel pharmocologiquement acceptable, si présent, est du citrate de sodium, du lactate de sodium, de l'hydrogénophosphate de disodium ou de l'hydrogénocarbonate de sodium.

8. Préparation de l'une quelconque des revendications précédentes dans laquelle le sel pharmacologiquement acceptable, si présent, est du chlorure de sodium.

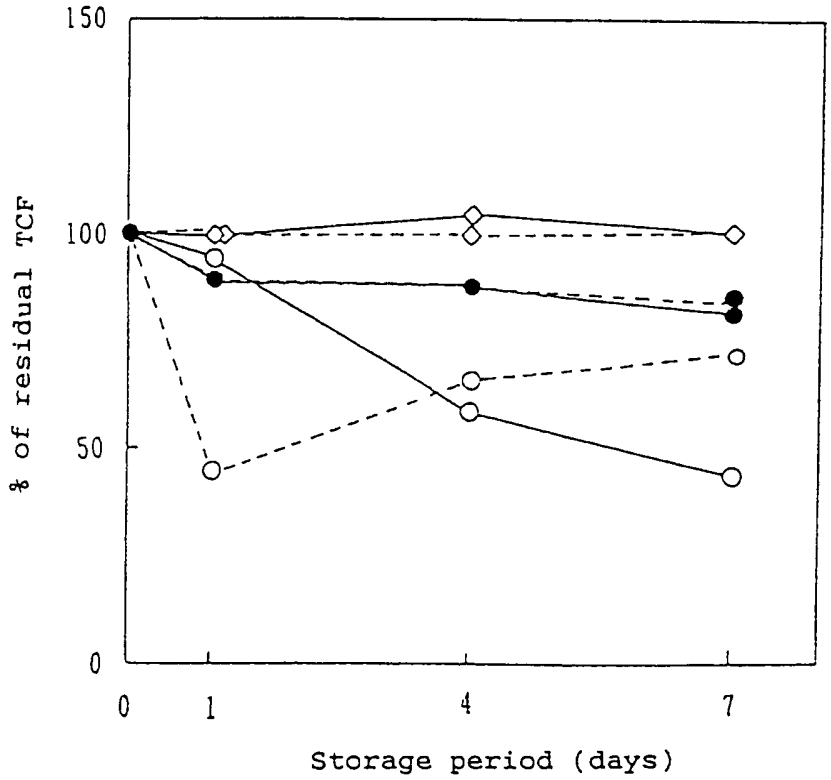
9. Préparation de l'une quelconque des revendications précédentes qui contient aussi un tensioactif, et un agent anti-adsorption ou un stabilisant ou une combinaison de deux quelconques ou plusieurs de ceux-ci.

10. Méthode pour produire les préparations des revendications 1-9, la méthode comprenant combiner le TCF avec un acide aminé basique ou sel de celui-ci.

11. Méthode de la revendication 10 dans laquelle l'acide aminé basique ou sel est de la lysine ou de l'arginine, ou un sel de lysine ou d'arginine.

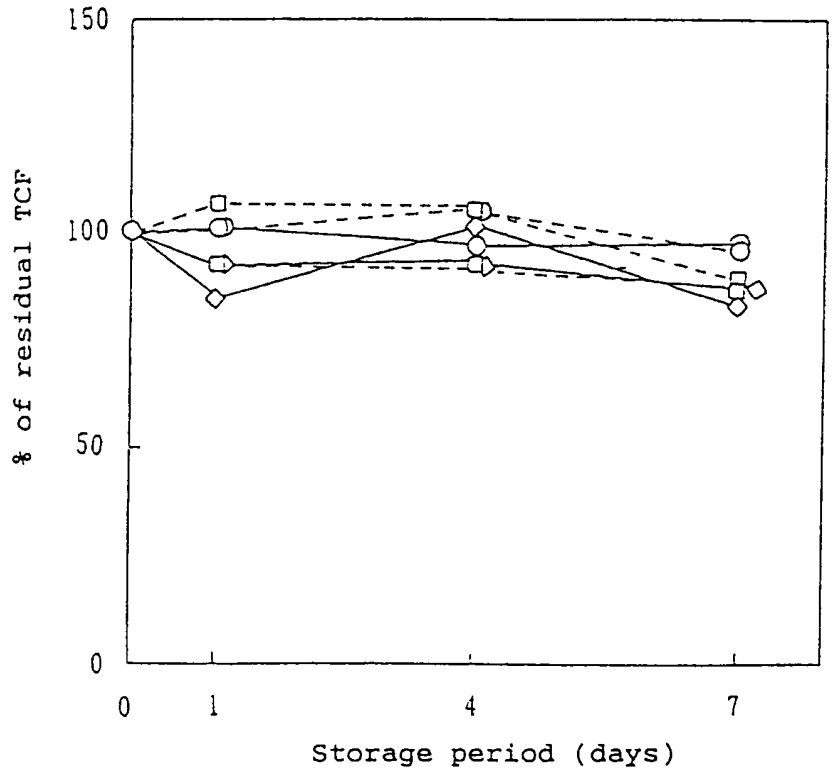
12. Méthode de la revendication 11 dans laquelle le sel pharmacologiquement acceptable, si présent, est du citrate de sodium, du lactate de sodium, de l'hydrogénophosphate de disodium, de l'hydrogénocarbonate de sodium ou du chlorure de sodium.

Fig. 1



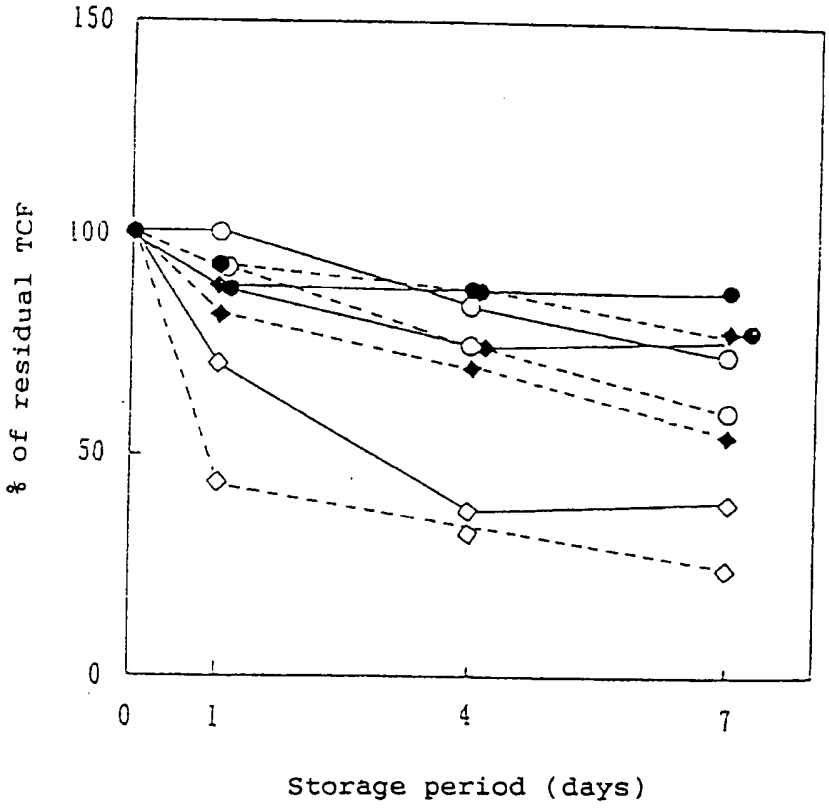
---: 5 °C
——: 20 °C
○: 0.15 M NaCl, pH 7.0
◇: 0.3 M NaCl, pH 7.0
●: 0.15 M NaCl, pH 6.0

Fig. 2



---: 5 °C
——: 20 °C
○ : 3.5 % L-arginine HCL
: 0 M NaCl
: 0 % D-mannitol, pH 7.0
◇ : 1.75 % L-arginine HCL
: 0.075 M NaCl
: 0 % D-mannitol, pH 7.0
□ : 1.75 % L-arginine HCL
: 0 M NaCl
: 2.5 % D-mannitol, pH 7.0

Fig. 3



---: 5 °C
——: 20 °C
○: 0.5 % HSA
: 0.15 M NaCl
: 0 % D-mannitol, pH 7.0
◇: 0.5 % HSA
: 0.075 M NaCl
: 2.5 % D-mannitol, pH 7.0
●: 0.5 % HSA
: 0.15 M NaCl
: 0 % D-mannitol, pH 6.0
◆: 0.5 % HSA
: 0.075 M NaCl
: 2.5 % D-mannitol, pH 6.0